In vitro anti-proliferative activities of *Aloe perryi* flowers extract on human liver, colon, breast, lung, prostate and epithelial cancer cell lines

Mai Mohammad Al-Oqail, Amina El-Shaibany, Ebtesam Al-Jassas, Ebtesam Saad Al-Sheddi, Shaza Mohamed Al-Massarani and Nida Nayyar Farshori*
Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Abstract: Natural products, especially plant extracts have offered vast opportunities in the field of drug development due to its chemical diversity. The genus *Aloe* has for long been used for medicinal purposes in different parts of the world. The present study was designed to investigate the phytochemicals and anti-cancer potential of *Aloe perryi* flowers. The phytochemical analysis revealed the presence of carbohydrates, glycosides, phytosterols, phenols, flavonoids and proteins. While alkaloids and saponins were absent. The percentage inhibition of various extracts (*viz.* petroleum ether, chloroform, ethyl acetate, butanol and aqueous) of *A. perryi* flowers on seven human cancer cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEp-2 and HeLa) has been evaluated using MTT assay. All the extracts significantly inhibit the proliferation of cancer cells in a concentration-dependent manner. The petroleum ether extract was most active, where the inhibition was recorded as 92.6%, 93.9%, 92%, 90.9%, 88.9%, 82% and 85.7% for HepG2, HCT-116, MCF-7, A-549, PC-3, HEp-2 and HeLa cells, respectively. The results also revealed that HCT-116 cells were more sensitive among all the cell lines studied.

Keywords: *Aloe perryi*, phytochemicals, cancercell lines, cytotoxicity, viability assay.

INTRODUCTION

Cancer is symbolized by an unregulated cell growth. The most common types of cancers being lung cancer, stomach cancer, liver cancer, colorectal cancer and breast cancer (WHO, 2010). Globally cancer continues to fig. among the leading causes of death with approximately 8.2 million cancer related deaths in 2010 (WCR, 2014). In the next two decades the demographics of population will change, this means that even if the current cancer rate remains the same, the incidence of 12.7 million new cases in 2008 will mount to 21.4 million by 2030 (GLOBOCAN, 2010). With such alarming figs. in the expected rise of cancer cases, several approaches are looked upon to develop an effective cure for this deadly disease (Neidle and Thurston, 2005). Although various advancements in the prevention and treatment of cancer have occurred, the successful treatment remains a challenge. Chemotherapy is a common treatment that has proved useful in a number of cancer cases including breast, colorectal, pancreatic, osteogenic sarcoma, testicular, ovarian and certain lung cancers (DeVita Jr and Chu, 2008). However, poor selectivity and toxicity limits the use of chemotherapy. Therefore, the advancements in identifying new drugs with higher selectivity and less toxicity have gained momentum. Consequently, the exploration of herbal therapies to identify novel hits and leads has increased (Vickers, 2004).

For thousands of years, plants are an important source of medicine in pharmaceutical biology. As per WHO, even today, 80% of population relies on traditional medicine (Yates, 2002). The genus aloe (family Asphodelaceae) is a collection of flowering succulents consisting of over 500 known species including *Aloe vera*, *Aloe barbadensis*, *Aloe ferox*, *Aloe chinensis*, *Aloe indica*, *Aloe perryi* etc. *A. perryi*, also known as Perry's aloe is endemic to Island of Socotra in Yemen. Its natural habitat is the dry rocky areas. Chemically Aloe possesses various pharmacologically important compounds such as essential oils, alkaloids, amino acids, anthroquinone glycosides, glycoproteins, vitamins, minerals and lectins (Al-Dubai and Al-Khulaidi, 1996; Atherton, 1997; Bazeb, 2002; Jia *et al.,* 2008; Naser 2005). Aloe species originating from the Arabian Peninsula are well documented for their medicinal usage and out of this *A. perryi* has the widest usage. Traditional uses of *A. perryi* include wound healing, burns and topical treatment of skin diseases (Al-Fatimi *et al.,* 2005). Several researchers have also revealed the role of *A. perryi* in the treatment of eye infections, stomach ailments, constipation and malaria (Mothana *et al.,* 2012; Mothana *et al.,* 2009). Antimicrobial effect of *A. perryi* is also reported (Awadh Ali *et al.,* 2001).

However, despite the widespread usage of *A. perryi* in traditional medicine, our literature survey reveals only a few reports on its cytotoxicity (Mothana *et al.,* 2012; Mothana *et al.* 2009). Furthermore, no report was available on the phytochemicals present and cytotoxic

**Corresponding author:* e-mail: nidachem@gmail.com

potential of flowers of *A. perryi*. Thus, this study aims to explore the anti-proliferative potential of various extracts (viz. petroleum ether, chloroform, ethyl acetate, butanol and aqueous) of *A. perryi* flowers against several human cancer cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEp-2 and HeLa) and their screening for phytochemicals present.

MATERIAL AND METHODS

Chemicals and consumables

Dulbecco's Modified Eagle's Medium (DMEM) an all other chemicals, solvents and reagents were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Trypsin-EDTA solution, and antibiotic/antimycotic solution and Fetal bovine serum (FBS) were purchased from GIBCO® Invitrogen, Life Technologies, USA. Culture wares and consumable used in this study were procured from Nunc, Denmark.

Cell culture

Human hepatocellular carcinoma (HepG2), human colon cancer (HT-116), human breast cancer (MCF-7), human lung adenocarcinoma (A-549), human prostate cancer (PC3), human epithelial carcinoma (HEp-2) and human cervical cancer (HeLa) cell lines were cultured in DMEM, supplemented with 10% FBS, 0.2% sodium bicarbonate, and 1% antibiotic/antimycotic solution. Cells were grown in 5% CO_2 at 37^0C in high humid atmosphere.

Plant material and extraction

The fresh flowers of *A. perryi* were collected during the flowering stage in May-June, 2013, from Island of Socotra, Yemen. A plant taxonomist in the Department of Botany, Faculty of Science, Sana'a University, Yemen authenticated the flowers and a voucher specimen (#4469) is deposited in the herbarium Pharmacognosy Department, Sana'a University.

The *A. perryi* flowers dried under shade, powdered coarsely and stored in airtight container for further use. The powdered *A. perryi* flowers (1.5 kg) were extracted with methanol (3 × 10L) at room temperature using cold maceration procedure. The combined methanol extract was concentrated under reduced pressure to obtain a thick gummy mass. It was suspended in water and successively extracted with petroleum ether (A), chloroform (B), ethyl acetate (C), *n*-butanol (D), and remaining water soluble fraction (E).

Phytochemical screening

The crude extracts of *A. perryi* were subjected to preliminary screening for the presence of active secondary metabolites (alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, flavonoids and proteins). Each plant extract was tested with specific chemical reagents according to the standard procedures (Tiwari *et al.*, 2011).

Experimental design

Different cell lines tested in this study were used to evaluate the cytotoxic effects of *A. perryi* flowers extract. All the cells were treated with different concentrations (1.56-50 µg/mL) of extract for 24 h. After treatment, the anti-proliferative activities was determined using 3-(4,5-dimethylthiazol-2-yl),5-biphenyl tetrazolium bromide (MTT) assay. Untreated control sets were run under identical condition.

Drug solutions

The extracts were not completely soluble in aqueous medium; therefore, the stock solutions of all the extracts were prepared in dimethylsulphoxide (DMSO) and diluted in culture medium to reach the desired concentrations. The concentration of DMSO in culture medium was not more that 0.1% and this medium was used as control.

Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the tested cell lines were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 µl of growth medium. Fresh medium containing different concentrations of the extracts was added after 24 h of seeding. Serial two-fold dilutions of the extracts were added to confluent cell monolayers dispensed into 96-well, flat-bottomed micro titer plates (Falcon, NJ, USA) using a multi channel pipette. The micro titer plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. After the end of incubation period, the viable cells yield was determined by a colorimetric method. In brief, the media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Micro plate reader (TECAN, Inc.), using a test wavelength of 590 nm. The absorbance is proportional to the number of surviving cells in the culture plate. All the results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell viability of each tested compound was calculated (Mosmann, 1983; Wilson, 2000).

Data analysis

The percentage cell viability was calculated using the Microsoft Excel®. Percentage cell viability was calculated as follows:

$$\%\,\text{Cell viability} = \frac{\text{Mean Abs of control}\;\;\text{Mean Abs of test metabolite}}{\text{Mean Abs control}} \times 100$$

Where: Abs: absorbance at 590 nm.

The 50% cell inhibitory concentration (IC_{50}), the concentration required to kill or cause visible changes in 50% of intact mammalian cells, was estimated from graphic plots. STATA statistical analysis package was used for the dose response curve drawing in order to calculate IC_{50}.

RESULTS

Table 1 represents the various phytochemicals present in different extracts of *A. perryi*. The petroleum ether and chloroform extracts contain glycosides, phytosterols and proteins and amino acids. The ethyl acetate extract contains phytosterols and flavonoids. The butanol extract contains phenols and flavonoids. The aqueous extract contains glycosides, phytosterols and flavonoids.

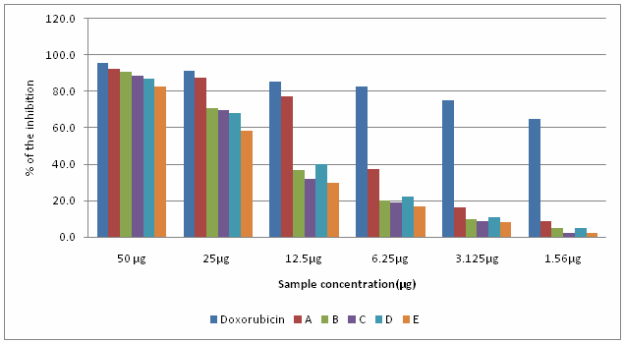

Fig. 1: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against HepG2 cell line.

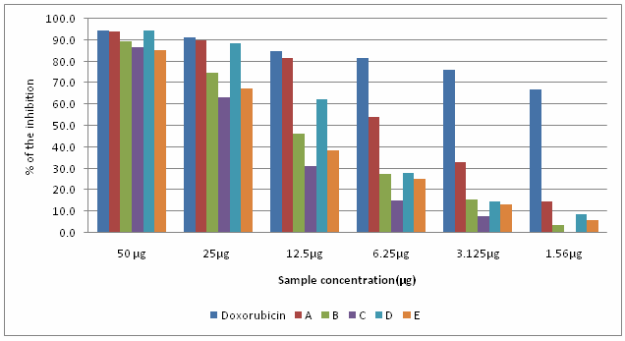

Fig. 2: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against HCT-116 cell line.

The percentage inhibition of petroleum ether, chloroform, ethyl acetate, butanol and aqueous extracts of flowers of *A. perryi* on different cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEp-2 and HeLA cell lines) has been evaluated using standard MTT assay. All the cell lines have been treated with increasing concentrations (1.56-50 µg/ml) for 24 h. The results obtained are shown in figs. 1-7. The results clearly show that all extract of *A. perryi* significantly inhibit the proliferation of cancer cells in a concentration-dependent manner. Moreover, the highest concentration of petroleum ether (50 µg/ml) show the highest inhibition in proliferation in all cell lines, where the inhibition was recorded as 92.6%, 93.9%, 92%, 90.9%, 88.9%, 82% and 85.7% for HepG2, HCT-116, MCF-7, A-549, PC-3, HEp-2 and HeLa cells, respectively. Furthermore, the results obtained, for all the cell lines also revealed that there was no significant effect on percentage inhibition at 1.56 µg/ml treated cells. Additionally, it can be clearly observed that A-549, PC-2 and HEp-2 cells exposed to 1.56 µg/ml of all solvent extracts did not show any significant difference in the percentage inhibition in the proliferation (figs. 4-6). On the other hand, HeLA cells also showed no inhibition when cells were treated with a concentration ranging from 3.125 to 12.5µg/ml of ethyl acetate and aqueous extract of *A. perryi* (fig. 7). The results clearly showed that HCT-116 cells were more sensitive among all the cell lines studied. On the other hand among the extracts, the highest percentage of inhibition for petroleum ether extract was found in HCT-116 cell line with the (IC_{50}=5.61 µg/ml), followed by HeLa (IC_{50}=5.83 µg/ml), A-549 (IC_{50}=7.54 µg/ml), MCF-7 (IC_{50}=7.88 µg/ml), HepG2 (IC_{50}=8.2 µg/ml), PC-3 (IC_{50}=9.51 µg/ml) and HEp-2 (IC_{50}=10.1 µg/ml) (table 2).

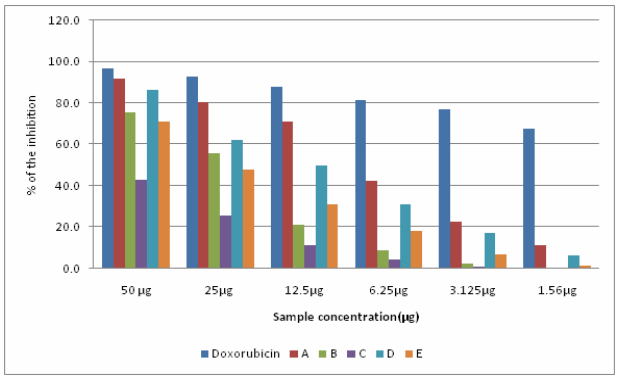

Fig. 3: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against MCF-7 cell line.

DISCUSSION

Natural products are being used since the beginning of human history for the medical purposes to treat various diseases including cancer (Al-oqail *et al.*, 2013). Many chemo preventive medicines are the molecules derived from the plant materials or there synthetic analogues (Solowey *et al.*, 2014). Plant territory has been the most important source and currently, ~60% of drugs used to treat the cancer have been isolated from natural products (Gordaliza, 2007), such as vincristine and vinblastine from Catharanthus roseus (Johnson *et al.*, 1963), camptothecins from Camptotheca acuminate (Wall *et al.*,

1966), taxol and docetaxel from Taxus brevifolia (Wani *et al.*, 1971). Vegetables and fruits have also been known to reduce the risk of cancer in humans (Chen *et al.*, 2006; Moon *et al.*, 2011). Some of the isolated compounds have exhibited anticancer potential with the low toxicity as compared to conventional drugs, eg. meisoindigo, isolated from the Chinese plant Indigofera tinctoria and flavopiridol, isolated from the Indian tree Dysoxylum binectariferum (Saklani and Kutty, 2007).

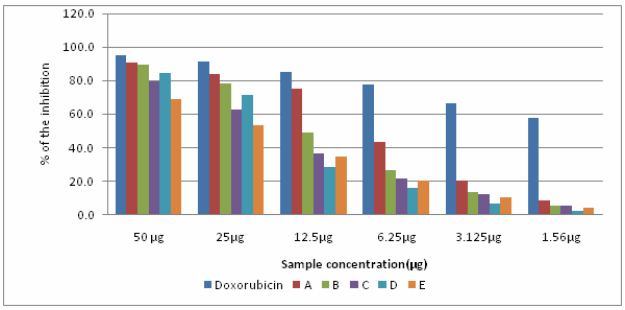

Fig. 4: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against A-549 cell line.

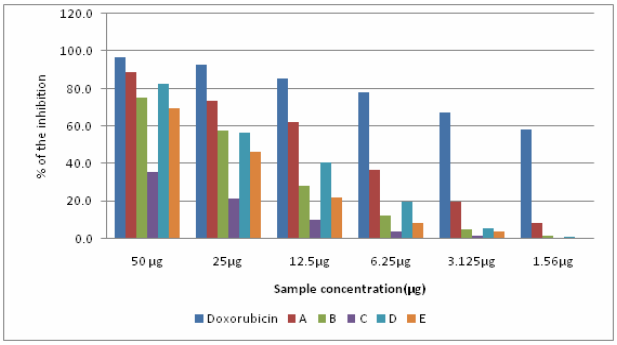

Fig. 5: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against PC-3 cell line.

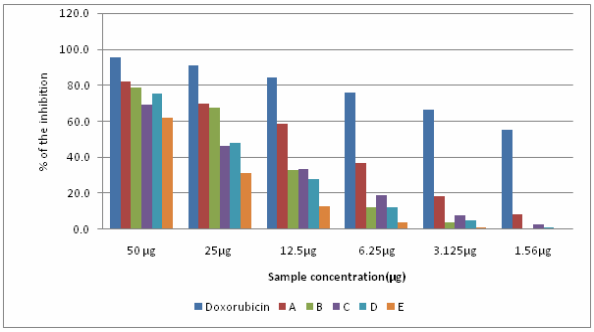

Fig. 6: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against HEp-2 cell line.

With the hypothesis that *A. perryi* flower extract might contain various antitumor agents that could be incredibly useful in killing the different human cancer cells, this study, was designed to screen the phytochemicals present and examine the effects of petroleum ether, chloroform, ethyl acetate, butanol and aqueous extracts of *A. perryi* on different human cancer cell lines, i.e. hepatocellular carcinoma (HepG2), colon cancer (HT-116), breast cancer (MCF-7), lung adenocarcinoma (A-549), prostate cancer (PC3), epithelial carcinoma (HEp-2) and cervical cancer (HeLa).

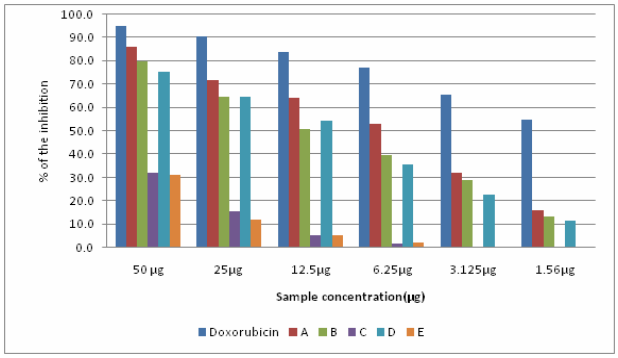

Fig. 7: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against HeLa cell line.

The phytochemical screening of various extracts of *A. perryi* revealed the presence of some secondary metabolites such as glycosides, phytosterols, phenols, flavonoids and proteins and amino acids as shown in table 1. These phytochemicals detected are known to have medicinal importance as anticancer, antibacterial, analgesic, anti-inflammatory, antitumor and antiviral agents (Cai *et al.* 2004, Miliauskas *et al.* 2004). The cytotoxicity evaluation revealed that increasing the concentration of *A. perryi* extract greatly inhibit the cell proliferation of different cell lines in a concentration dependent manner, when cells were treated with 1.56 to 50 µg/ml for 24 h. Our results are in well accordance with those of Al-Oqail *et al.* (2013), who obtained a dose-dependent response of different concentrations on HEp2, MCF-7, WISH and Vero cells. Our results also demonstrated that HCT-116 cells were more sensitive among all the cell lines studied and among the extracts, the highest percentage of inhibition for petroleum ether extract was found in HCT-116 cell line with the (IC_{50}=5.61 µg/ml), followed by HeLa (IC_{50}=5.83 µg/ml), A-549 (IC_{50}=7.54 µg/ml), MCF-7 (IC_{50}=7.88 µg/ml), HepG2 (IC_{50}=8.2 µg/ml), PC-3 (IC_{50}=9.51 µg/ml) and HEp-2 (IC_{50}=10.1 µg/ml). These kind of variation among different cell lines have also been reported previously by Heo *et al.* (2014), who have reported anticancer effects of plant extract on HEK-293, HCT-116, HeLa, MCF-7, Hep3B, SNU- 1066 and SNU-601 cell lines. In other study, differential cytotoxic response towards different cancer cell lines (HeLa, HepG2, MCF-7, CACO-2 and L929) have also been reported and concluded that the plant extract effectively inhibit the proliferation of cells depending on the extract concentration as well as cell types (Elsayed *et al.*, 2015). Our results are also in

Table 1: Phytochemical analysis of various extracts of *Aloe perryi* flower.

Chemical Constituents	Chemical Tests	Extracts[a]				
		A	B	C	D	E
Carbohydrates	Molisch's Test	-	-	-	-	+
Glycosides	Modified Borntrager's Test	+	+	-	-	-
Alkaloids	Mayer's Test	-	-	-	-	-
Phytosterols	Libermann Burchard's Test	+	+	+	-	+
Phenols	Ferric Chloride Test	-	-	-	+	-
Flavonoids	Alkaline Reagent Test	-	-	+	+	+
Proteins & Amino Acids	Xanthoproteic Test	+	+	-	-	-
Saponins	Froth Test	-	-	-	-	-

[a]: A, Petroleum ether; B, Chloroform; C, Ethyl acetate; D, n-Butanol; E, Aqueous; +, present; -, Absent.

Table 2: IC_{50} values obtained for each of the extracts in different human cancer cell lines.

Tumor Cell Lines	$IC_{50}$of Petroleum Ether extract (µg)	$IC_{50}$of Chloroform extract (µg)	IC_{50} of Ethyl Acetate extract (µg)	IC_{50} of Butanol Extract (µg)	$IC_{50}$of Aqueous extract (µg)
HepG-2	8.2	17.3	18.5	16.9	21.5
HCT-116	5.61	14.1	19.8	10.3	17.5
MCF-7	7.88	22.9	>50	12.7	27.4
A-549	7.54	13.0	18.8	18.8	22.6
PC-3	9.51	21.7	>50	19.7	29.4
HEp-2	10.1	18.7	29.1	26.7	40.1
HeLa	5.83	12.1	>50	11.1	>50

agreement with the previous findings, where plant extracts decrease the cell viability in human breast cancer (T47D) cells, due to the sensitivity of cancerous cells towards the death flavanoids (Abdolmohammadi *et al.*, 2008). Furthermore, growth inhibitory effect of certain constituents of plant also have been shown in human uterus carcinoma (HeLa), murine melanoma (B16F10) cells, human gastric adenocarcinoma (MK-1) (Fujika *et al.*, 1999) and in other human cancer cell lines (Kim *et al.*, 2002; Kumi-Diaka and Butler, 2000; Farshori *et al.*, 2013; Farshori *et al.*, 2014). This growth inhibitory activity might be due to the ability of plant extracts to inhibit the DNA synthesis as measured by the incorporation of tritiated thymidine into cells (Worthen *et. al., 1998*), which leads to cell death (Watson & Preedy, 2010).

CONCLUSION

In conclusions, this study provides a phytochemical analysis and preliminary screening for anti-proliferative activity of various *A. perryi* extracts on different cancer cell lines. We have shown that different extracts of *A. perryi* significantly inhibit the growth of various cancer cell lines (HepG2, HT-116, MCF-7, A-549, *PC3,* HEp-2 and HeLa) in a concentration-dependent manner. Among the all extracts, petroleum ether have shown more activity and HCT-116 cells were more sensitive, followed by HeLa, A-549, MCF-7, HepG2, PC-3 and HEp-2 cells. In addition, the presence of phytochemicals such as phytosterols, phenols, flavonoids, proteins and glycosides has been confirmed. Further investigations are required to understand the possible mechanism(s) of action of these extract on various cancer cells and isolation of active phytochemicals.

ACKNOWLEDGEMENT

This research project was supported by a grant from the "Research Centre of the Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

REFERENCES

Abdolmohammadi MH, Fouladdel Sh, Shafiee A, Amin Gh, Ghaffari SM and Azizi E (2008). Anticancer effects and cell cycle analysis on human breast cancer T47D cells treated with extracts of Astrodaucus persicus (Boiss.) Drude in comparison to doxorubicin. *DARU.*, **16**: 112-118.

Al-Dubai AS and Al-Khulaidi AA (1996) Medical and aromatic plants of Yemen (In Arabic). Obadi center for studies and publishing, Sana'a, Yemen

Al-Fatimi M, Friedrich U and Jenett-Siems K (2005). Cytotoxicity of plants used in traditional medicine in Yemen. *Fitoterapia.*, **76**: 355-358.

Al-Oqail MM, Farshori NN, Al-Sheddi ES, Musarrat J, Al-Khedhairy AA and Siddiqui MA (2013). *In vitro* cytotoxic activity of seed oil of fenugreek against various cancer cell lines. *Asian Pac. J. Cancer Prev.*, **14**: 1829-1832.

Atherton P (1997). The essential Aloe vera. Newport Pagnell: Mill Enterprises.

Awadh Ali NA, Jülich WD, Kusnick C and Lindwquist U (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J. Ethnopharmacol*., **74**: 173-179.

Bazeeb AS (2002). The medicinal plants in Yemen (3rd edn. ed). EL-Ershad Press: Sana'a, Yemen.

Cai YZ, Luo Q, Sun M and Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.*, **74**: 2157-2184.

Chen TJ, Jeng JY, Lin CW, Wu CY and Chen YC (2006). Quercetin inhibition of ROS-dependent and independent apoptosis in rat glioma C6 cells. *Toxicology*, **223**: 113-126.

DeVita VT Jr and Chu E (2008). A History of Cancer Chemotherapy. *Cancer Res*., **68**: 8643-8653.

Elsayed EA, Sharaf-Eldin MA and Wadaan M (2015). *In vitro* evaluation of cytotoxic activities of essential oil from Moringa oleifera seeds on HeLa, HepG2, MCF-7, CACO-2 and L929 cell lines. *Asian Pac. J. Can. Prev.*, **16**: 4671-4675.

Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, and Siddiqui MA (2014). Cytotoxicity Assessments of Portulaca oleraceaand Petroselinum sativum Seed Extracts on Human Hepatocellular Carcinoma Cells (HepG2). *Asian Pac. J Can. Prev.*, **15**: 6633-6638.

Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA and Siddiqui MA (2013). Anticancer activity of Petroselinum sativum seed extracts on MCF-7 Human Breast cancer cells. *Asian Pac. J. Cancer Prev.*, **14**: 5719-5723.

Fujika T, Furumi K, Fujii H, Okabe H, Mihashi K, Nakano Y, Matsunaga H, Katano M and Mori M (1999). Antiproliferative constituents from umbelliferae plants. A new furanocoumarin and falcarindiol furanocoumarin ethers from the root of angelica japonica. *Chem. Pharm. Bull.*, **47**: 96-100.

GLOBOCAN (2010). Available at (http://www.iarc.fr/en/media-centre/iarcnews/2010/ GLOBOCAN2008.pdf)

Gordaliza M (2007). Natural products as leads to anticancer drugs. *Clin. Transl. Oncol.*, **9**: 767-776.

Heo BG, Park YJ, Park YS, Bae JH, Cho JY, Park K, Jastrzebski Z and Gorinstein S (2014). Anticancer and antioxidant effects of extracts from different parts of indigo plant. *Indus. Crops Prod.*, **56**: 9-16.

Jia Y, Zhao G and Jia J (2008). Preliminary evaluation: the effects of *Aloe ferox* Miller and *Aloe arborescens* Miller on wound healing. *J. Ethnopharmacol*., **120**: 181-189.

Johnson IS, Armstrong JG, Gorman M and Burnett Jr JP (1963). The vinca alkaloids: A new class of oncolytic agents. *Cancer Res.*, **23**: 1390-1427.

Kim YJ, Liu RH, Rychlik JL and Russell JB (2002). The enrichment of a ruminal bacterium (Megasphaera elsdenii YJ-4) that produces the trans-10, cis-12 isomer of conjugated linoleic acid. *J. Appl. Microbiol.*, **92**: 976-982.

Kumi-Diaka J and Butler A (2000). Caspase-3 protease activation during the process of genistein induced apoptosis in TM4 cells. *Biol. Cell*, **92**: 115-124.

Miliauskas G, Venskutonis PR and Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, **85**: 231-237.

Moon JY, Mosaddik A, Kim H, Cho M, Choi HK, Kim YS and Cho SK (2011). The chloroform fraction of guava (Psidium cattleianum sabine) leaf extract inhibits human gastric cancer cell proliferation via induction of apoptosis. *Food Chem.*, **125**: 369-375.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**: 55-63.

Mothana RA, Al-Musayeib NM, Matheeussen A, Cos P and Maes L (2012). Assessment of the *in vitro* antiprotozoal and cytotoxic potential of 20 selected medicinal plants from the island of Soqotra. *Molecules*, **17**: 14349-14360.

Mothana RA, Lindequist U, Gruenert R and Bednarski PJ (2009). Studies of the *in vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island of Soqotra. *BMC Complement Altern. Med.*, **9**: 7.

Naser AA (2005) Natural Pharmacy (1st end. ed) Obadi center for studies and publishing. Sana'a, Yemen.

Neidle S and Thurston DE (2005). Chemical approaches to the discovery and development of cancer therapies. *Nat. Rev. Cancer*, **5**: 285-296.

Saklani A and Kutty SK (2008). Plant-derived compounds in clinical trials. *Drug discovery today*, **13**: 161-171.

Solowey E, Lichtenstein M, Sallon S, Paavilainen H, Solowey E and Lorberboum-Galski H (2014). Evaluating medicinal plants for anticancer activity. *Sci. World J.*, **721402**: 1-12.

Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H (2011). Phytochemical screening and Extraction: A Review. *Internationale. Pharmaceutica. Sciencia.*, **1**: 98-106.

Vickers A (2004). Alternative cancer cures "unproven" or "disproven". *CA: Cancer J. Clin.*, **54**: 110-118.

Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT and Sim GA (1966). Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Camptotheca acuminata. *J. Amer. Chem. Soc.*, **88**: 3888-3890.

Wani MC, Taylor HL, Wall ME, Coggon P and McPhail AT (1971). Plant antitumor agents VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J. Amer. Chem. Soc.*, **93**: 2325-2327.

Watson RR and Preedy VR (Eds.). (2010). Bioactive foods and extracts: Cancer treatment and prevention. CRC Press. 2010.

WHO (2010). Cancer. (http://www.who.int/mediacentre/factsheets/fs297/en/).

Wilson AP (2000). Cytotoxicity and viability assays in animal cell culture: A Practical Approach, 3rd ed. (ed. Masters, J. R. W.) Oxford University Press.

World cancer report (WCR) (2014). (http://www.iarc.fr/en/publications/books/wcr/wcr-order.php).

Worthen D, Ghosheh O and Crooks P (1998). The *in vitro* anti-tumor activity of some crude and purified components of black seed, *Nigella sativa* L. *Anticancer Res*., **18**: 1527-32.

Yates A (2002). Yates Garden Guide, Harper Collins Australia, Australia.